position with respect to the macromolecule. The structure of the 4WJ was compared with FRET restrained structures of related RNA 3WJs, where one stem was removed. The types of 3WJs were studied: (I) without bulges, (II) with a small bulge (two unpaired nucleotides) and (III) with larger bulge (5 unpaired nucleotides). In conclusion the overall geometry of the RNA helices depends drastically on the junction type.

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[2] Sindbert, S., et al.; J. Am. Chem. Soc. **133**, 2463-2480 (2011)
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143-Plat
Observation of Intrinsic Conformational Dynamics of TPP Riboswitch and its Structural Transition Induced by TPP Binding
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A riboswitch is a non-coding region of an mRNA which recognizes a specific metabolite and regulates the expression of the mRNA itself. They are composed of two domains: an aptamer domain which recognizes a metabolite, and downstream expression platform. It is believed that metabolite binding to the aptamer domain induces a structural change of the riboswitch, signaling ''on'' or ''off'' for the transcription or translation of the mRNA.Recent publications of a number of high-resolution structures of ligand-bound aptamer domains (halo-form) greatly contributed to our understanding of how the riboswitches interact with their natural ligands at the atomic-level. However, little is known about the nature of the unbound form of riboswitches (apo-form), and how conformational changes of riboswitches is induced by binding of a ligand to the aptamer domain.We used fluorescence resonance energy transfer (FRET) to study conformational dynamics of the Escherichia coli thiM TPP (thiamine pyrophosphate) riboswitch. We found that even though the apo-aptamer dynamically samples a closed conformation resembling the halo-aptamer, both the open form and the closed form recognize the ligand with equal preference. And the final structural transition of the riboswitch induced by the ligand is also observed. Both indicate that the ligand is recognized via the induced-fit mechanism. Furthermore, the role of Mg ions on the ligand recognition mechanism will be also discussed.

144-Plat
Effect of Non-Canonical Structures on the DNA Duplex
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DNA is constantly being modified by exogenous and endogenous agents, leading to covalent changes to its structure and, if left unrepaired, to permanent changes in sequence. We use a wide variety of biophysical techniques to examine the effect on DNA structure and dynamics of the common base lesion 8-oxoguanine, the cis-syn thymine dimer lesion, single-base mismatches, and bubbles. The stability of duplex formation in the presence of these disturbances is explored using UV melting thermodynamics, calorimetry, and single-molecule force measurements. Duplex structure is analyzed using small molecule reactive probes, circular dichroism, and NOESY spectroscopy. NMR proton exchange experiments reveal locations of dynamic changes to the DNA duplex, including base pair opening and flexibility. By bringing a diverse set of techniques to bear on each non-canonical structure, we have shown the 8oxoG lesion to be surprisingly stable, while the thymine dimer lesion is significantly more labile. Even Hoogsteen-binding "wobble" mismatches can effect the kinetics and thermodynamics of duplex formation in surprising ways. These results complement and extend the information obtained by crystallographic methods to include dynamic and thermodynamic factors.

145-Plat
Single-Stranded DNA is not a Worm-Like Chain
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The structure of unfolded, flexible polyelectrolytes in solution, while an important subject for understanding various biological and technological phenomena such as RNA and protein folding and the self-assembly of DNA nanostructures, is poorly understood. While it is well-known and tested that semi-flexible polyelectrolytes, such as dsDNA, behave as Worm-Like Chains (WLC) with a scale-dependent electrostatic persistence length, validity of this model for flexible polyelectrolytes is doubtful. However, throughout the literature, data on flexible polyelectrolytes are naively fit to WLC models due to the simplicity of the analytical expressions. Here, we examine the validity of the WLC model for flexible polyelectrolytes using single-molecule force spectroscopy on ssDNA. We find that the force-extension data cannot be adequately fit to the Marko-Siggia prediction for the WLC even after accounting for electrostatic effects. Rather, our data reveal a self-avoiding walk regime consistent with scaling predictions at low force followed by a regime where the extension scales as a logarithm with force over a broad range of moderate forces and monovalent salt concentrations. Further, we run molecular dynamics simulations on a bead-spring model polyelectrolyte under tension and reproduce this logarithmic behavior in monovalent salt, indicating that it is indeed a general behavior for flexible polyelectrolytes. Examining the structure factor of the simulated polymer reveals a highly wrinkled, ion-stabilized structure at length scales smaller than a Debye length which defies characterization as a WLC. Addition of divalent salt to either the simulation or experiment results in enhanced flexibility indicating increased wrinkling or polymer ''wrapping'' around the divalent ions.

146-Plat
Biophysical Modeling of Nucleic Acid Nanostructure Solution Shape and Stability
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Programmed self-assembly of RNA and DNA provides a powerful approach to designing active, functional nanometer-scale structures for biomolecular science and technology. Scaffolded DNA Origami employs a long single-stranded DNA template to guide the hybridization of hundreds of shorter oligonucleotide staple strands to form twisted and bent double-stranded DNA structures of high precision and yield with well-defined mechanical properties. Biophysical models of nanostructure formation and stability are needed to inform the DNA Origami design process, which currently proceeds largely based on physical intuition and trial-and-error. Here we present a physics-based model for DNA Origami that incorporates double-stranded DNA mechanical properties in addition to screened electrostatic interactions on 3D solution shape and flexibility. Results are presented in detail for an eight-layer DNA Origami block designed on a square lattice (Ke et al., JACS 2009), whose 3D solution shape is computed under experimental folding conditions, revealing an undulating pattern of individual DNA double helices due to interhelix electrostatic repulsion. Effects of crossover spacing on solution shape and mechanical properties are also discussed.

147-Plat
Computational Studies on the Effects of DNA Methylation on its Structure and Dynamics
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DNA methylation occurs in CpG dinucleotides when a methyl group is added at the carbon 5 position of the C base ring, and it has an important role in regulating gene expression in higher eukaryotes. It is known that when cytosine bases are methylated, gene is silenced, but the precise role of methylated DNA in this process is still debated, and the mechanism by which methylation affects gene expression remains poorly understood.
One hypothesis is that the methylation state of a DNA segment can alter the positions of nucleosomes, which in turn, determine gene expression. Several studies point to a strong link between CpG methylation and nucleosome positioning, but these effects might be indirect because methylation influences the binding of other factors, which could in turn trigger nucleosome repositioning. Because methylation chemically modifies DNA, the methylation status of a DNA sequence could influence its flexibility and, thus, its affinity to the nucleosome. Recent experimental studies suggest that CpG methylation can decrease the ability of DNA to bend into the major groove at the methylated site, and can thereby influence nucleosome positioning.
To test the idea that methylation affects the ability of DNA to bend, we performed MD simulations of short DNA segments using an all atom force field. DNA conformations were characterized using the six rigid body base-pair step parameters, the minor groove width and the hybridization state. These properties were measured and compared for dsDNA segments in both the unmethylated and methylated states. In addition, all atom trajectories for both the cases were analyzed to extract a new set of parameters for a coarse grain model for DNA that can now be used to study the effects of methylation in larger DNA systems such as nucleosomes.

148-Plat
Thermodynamics and Kinetics of Stretched, Plectonemic and Melted DNA
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In most organisms DNA is negatively supercoiled, which plays an important role in a variety of cellular processes. Supercoiling also forms the driving force for DNA compaction into chromosomes. Structurally, DNA under negative torsion can stay in a linear stretched and twisted configuration, can transit into plectonemes or can unwind its helix to form bubbles of melted DNA. The

partitioning of these three states depends on both force and the degree of supercoiling. However, the coexistence of these three states has not been described yet. Here we present a statistical physics model to describe DNA extension in the three coexisting states by calculating the full partition function. We compare these results to the extension of short DNA molecules at subpiconewton force in a range of linking number densities, measured with magnetic tweezers. Real-time analysis of the extension of short DNA tethers at constant force and linking number directly shows the dynamics of melting bubble formation, which we describe as a function of force and twist. Our results provide a comprehensive picture of the structure of underwound DNA and may have important consequences for various biological processes, in particular the ones that depend on local DNA melting, such as the initiation of replication and transcription.

149-Plat
Tuning DNA Bending with Charged Nanoparticles: Molecular Simulations
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The interactions between DNA and charged nanoparticles have transpired into applications from biosensors to DNA-templated metallization. However, the process of non-specific DNA binding with NP is difficult to characterize and is not well understood. We performed molecular dynamics simulations to understand the mechanisms of DNA-NP interactions and observed that the ligand chemistry on the NPs has contrasting impact on the helical structure of DNA. All atom simulations show that uncharged NPs with -CH3 and -NH2 ligand end groups can only bind to the minor groove of DNA through hydrophobic interactions and do not induce a DNA helical distortion. On the other hand, charged NPs (from +6 to +60) can bind to both major and minor grooves of DNA. Moreover, while highly charged NPs wrap the DNA tightly, weakly charged NP can partially denaturate the DNA helix through a collective clustering behavior. Overall, we observed that by tuning the ligand chemistry and its density of GNP we can control the binding modes and the structural mechanics of DNA.

Platform: Protein Dynamics I

150-Plat
Conformational Analysis of Processivity Clamps Demonstrates that Protein Tertiary Structure Does Not Correlate with Dynamics
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Processivity clamps are critical for efficient DNA replication in all organisms. Whereas these clamp proteins can be either dimers or trimers, they all exhibit pseudo-six-fold symmetry. Each pseudo-monomeric domain is highly conserved in its tertiary structure even though the primary structures are divergent. We previously characterized the beta processivity clamp from *Escherichia coli* using hydrogen-deuterium exchange mass spectrometry (HXMS), which probes the solvent accessibility and hydrogen bonding of each backbone amide hydrogen, except those in prolines and at the first residue. We found that the three different domains within each monomer displayed different dynamics and that Domain I, which dissociates from Domain III to open the clamp, underwent partially cooperative local unfolding with a half-life of ~4 h. To determine how general our observations of a highly dynamic clamp protein were, we carried out a similar analysis using HXMS to characterize the dynamics of clamps from bacteriophage T4, the yeast *Saccharomyces cerevisiae*, archaeon *Thermococcus kodakarensis*, plant *Arabidopsis thaliana*, and human. *Thermococcus kodakarensis* and *Arabidopsis thaliana* both have two different PCNA clamp proteins that show slightly different dynamics. This is especially intriguing in the case of the two PCNA proteins from *Arabidopsis thaliana*, as the primary structures are 96% identical. The different clamps show a wide range of dynamic properties. Bacteriophage T4 gp45 shows high deuterium uptake and undergoes widespread local unfolding events with half-lives of approximately 5 min. In human PCNA, local unfolding is observed at the trimer interfaces with a half-life of about 1 h. The clamp proteins from bacteriophage T4, *Arabidopsis thaliana*, and human incorporate the most deuterium and are therefore the most dynamic. We find a striking range of dynamic properties of the clamp proteins despite their highly conserved tertiary structures.

151-Plat
FTIR and Femtosecond 2D-IR Spectroscopy of Azidohomoalanine-Labeled PDZ3 from PSD-95: Site-Specific Probing of Ultrafast Dynamics and Electrostatics in Proteins
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Vibrational spectroscopy is a highly sensitive tool to study the structure and function of proteins. The absorption frequency of particular functional groups such as azides or nitriles can be sensitive to even small changes in the electrostatic environment. We use the methionine surrogate azidohomoalanine as a spectroscopic probe, which can be incorporated site-selectively during protein synthesis. Here we present recent work on the 3rd PDZ domain of PSD-95, a protein domain of 10.7kDa involved in protein-protein-interaction and signaling processes. The PDZ domain has various features that make it a suitable target for testing the application of an intrinsic azide group as a probe for the electrostatic environment. The protein displays both alpha-helical and beta-sheet secondary structure elements and has a hydrophobic peptide binding pocket. We incorporated azidohomoalanine at six positions in different secondary structures, in the interior and at the surface of the protein, as well as close to the binding pocket. Static IR spectra of all mutants show a clear correlation of the azide absorption frequency with the hydrophobicity of the surrounding side chains, the positions of which are known from x-ray structures. Furthermore changes in hydrophobicity upon ligand binding can be monitored, not only in FTIR data, but also in time-resolved 2D-IR spectroscopy. Using ultrafast 2D-IR spectroscopy we are able to measure fluctuations in the protein environment around our local probe on a picosecond timescale. Our data show that already on this timescale differences in dynamics upon ligand binding are induced.

152-Plat
Neutron Scattering Studies of Green Fluorescent Protein, Nanosecond-Picosecond Dynamics
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We present a detailed analysis of the picosecond-to-nanosecond motions of green fluorescent protein (GFP) and its hydration water using incoherent and coherent neutron scattering and hydrogen/deuterium contrast. Our results reveal that while much of the dynamics in the hydrated protein are connected to motions of hydration water, there are significant differences in the dynamics of protein and its hydration water. On the picosecond-to-nanosecond timescale, the hydration water exhibits diffusive dynamics, while the protein motions are localized to less than ~3Å. The beta-barrel structure of GFP differs from previously studied globular proteins; which may explain the differences observed in the direct comparison of the atomic displacements (on a timescale of ~1ns) between GFP and lysozyme. We expand upon this and the recent Biophysical Journal article on this system with a more in depth analysis of the quasielastic spectra and a comparison of the data for GFP to current models for dry and hydrated protein motions. Finally, coherent scattering measurements allow us to comment on the cooperativity of protein and hydration water dynamics.

153-Plat
Co-Folding of the Histone Chaperone DAXX and H3.3/H4
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Histone chaperones mediate the assembly and disassembly of canonical and variant nucleosomes. Currently there is little known of the conformational changes that occur upon chaperone-histone binding, but these are likely to be key to understanding the mechanism of histone transfer on and off of DNA. For this study, we focus on the death domain-associated protein (Daxx), a chaperone that specifically recognizes the H3.3 histone variant, mediating its deposition into heterochromatin. Using hydrogen/deuterium exchange (H/DX) coupled with mass spectroscopy to measure polypeptide backbone dynamics, we have obtained biochemical evidence of a co-folding mechanism of the Daxx histone-binding domain (HBD) with the H3.3/H4 histone dimer. Monomeric DAXX[HBD] undergoes extremely rapid H/DX, exhibiting the behavior of an unfolded protein. Upon binding to H3.3/H4, both Daxx and the H3.3/H4 subunits are globally stabilized, as measured by H/DX protection of several orders of magnitude. The H/DX rates in the ternary complex are matched at contact points between all three subunits as revealed by crystallography (Elsässer et al., 2012, in press), suggesting that they sample unfolded/folded states with the same kinetics throughout the complex. Importantly, Daxx binding stabilizes the helices of a H3.3/H4 complex in which point mutations disrupt stability in